Cell Wall Inhibitors Not β-Lactams

Introduction

The antibiotics in this lesson are the antibiotics that mess with the cell wall or the cell membrane but are not β-lactams. This may feel like a bucket of "antibiotics not otherwise specified," and that's okay. The mechanism of action of these medications directs which organisms they influence, and there are no generalities to be made about this list. This lesson assumes you've read and mastered Bacteria #1: *Bacterial Structure and Introduction* and Antibacterials #2: *β-Lactam Cell Wall Inhibitors*, and so are comfortable with cell wall synthesis and the difference between Gram positives and Gram negatives, reviewed briefly in Figure 3.1.

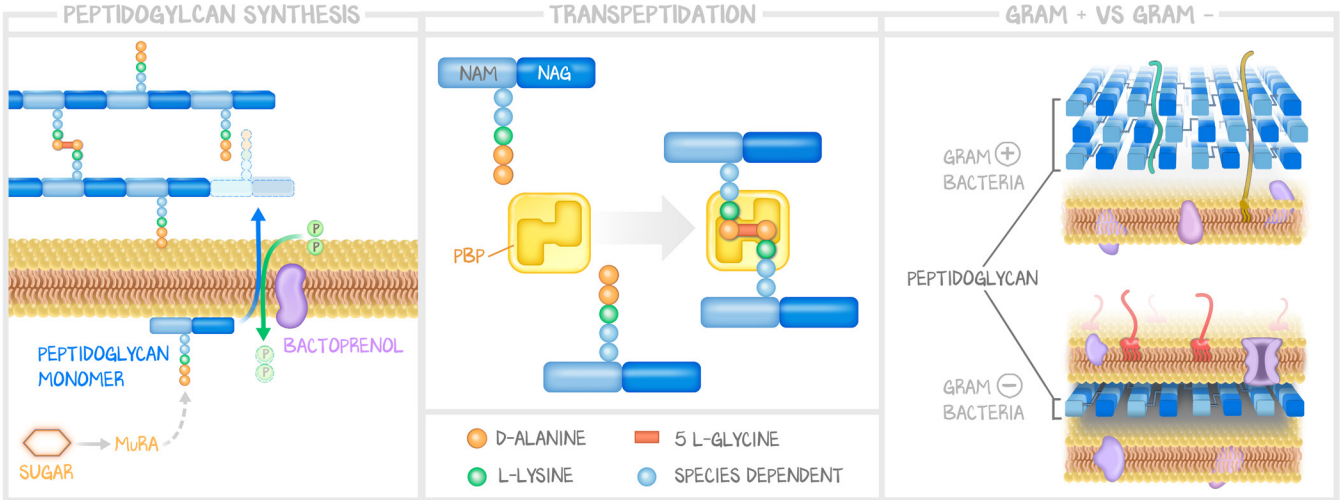

Figure 3.1: Normal Bacteria
Review of peptidoglycan synthesis and glycosylation. Transpeptidation, and the difference between Gram-positive and Gram-negative organisms.

This lesson covers vancomycin, bacitracin, fosfomycin, daptomycin, and polymyxins. By far the most important is vancomycin. It tells the story of clever scientists, clever bugs, and foolish clinicians who didn't listen to clever scientists. Poor antibiotic stewardship breeds antibiotic resistance faster than science can create new mechanisms of actions to compete with that resistance. Bacteria learn from our drugs and from each other. Vancomycin is a story of our failure, not our success.

Vancomycin

Staph. aureus is a clever bug with a really good β-lactamase. We created specific penicillins with side groups that made them extremely resistant to β-lactamase specifically to treat *Staph. aureus* infections. Those were the β-lactamase-resistant nafcillin and oxacillin. They work really well against methicillin-sensitive *Staph. aureus* (MSSA). But they don't work against any Gram negatives, because the way they became so resistant to β-lactamase was to increase their size, making them too big to fit through porins of the outer plasma membrane of Gram-negative bacteria. Logically, because strep species do not have an outer membrane, these β-lactams should work on strep as well. But the β-lactamase-resistant antibiotics should be used only for *Staph. aureus*, because strep species are usually susceptible to the others—and because administering a β-lactamase-resistant penicillin to strep also exposes endogenous *Staph. aureus* to an antibiotic that could teach it to become methicillin resistant. Developing resistance to these β-lactamase-resistant penicillins is really bad for humanity—we only took the risk with *Staph. aureus* because we had no alternative.

Methicillin is the laboratory version of nafcillin (the commonly used IV anti-staphylococcal penicillin) and oxacillin (the rarely used PO anti-staphylococcal penicillin). If *Staph. aureus* does not grow in methicillin, it is methicillin-sensitive *Staph. aureus* (MSSA), and nafcillin will work. If *Staph. aureus* does grow in methicillin, it is methicillin-resistant *Staph. aureus* (MRSA), and nafcillin will not work. It worked for a while.

But *Staph. aureus* is a clever bug. MSSA already had a β-lactamase that rendered all other β-lactams useless. Then, through poor antibiotic stewardship, we bred MRSA. MRSA arose through a **mutation of the penicillin-binding proteins**. Add that to the **uber β-lactamase**, and MRSA is invulnerable to all β-lactams.

MRSA is still a peptidoglycan-producing Gram-positive organism. It still has no outer membrane. It still has only a peptidoglycan cell wall. It's just that the enzymes in that cell wall (β-lactamase, PBPs) are so good at destroying or evading β-lactams that β-lactams cannot work. So, we created **vancomycin** with the intention of specifically treating MRSA. Let me say it again: **vancomycin treats MRSA**. It is not broad spectrum, it is not a big gun, it is the antibiotic we synthesized specifically to fight the bug we bred. Vancomycin is NOT a β-lactam. Vancomycin doesn't bind to any bacterial proteins. It binds to the **amino acid sequence** that penicillin-binding proteins (transpeptidase) bind to. The pentapeptide chain that will be used to cross-link peptidoglycan chains ends with **D-Ala-D-Ala**. Vancomycin **binds to D-Ala-D-Ala** with greater affinity than the penicillin-binding proteins do, creating a physical barrier, preventing PBPs from reaching their target, therefore making cross-linking impossible. The end result is the same as if we were using a penicillin—transpeptidation doesn't occur, the cell wall falls apart, the cell lyses.

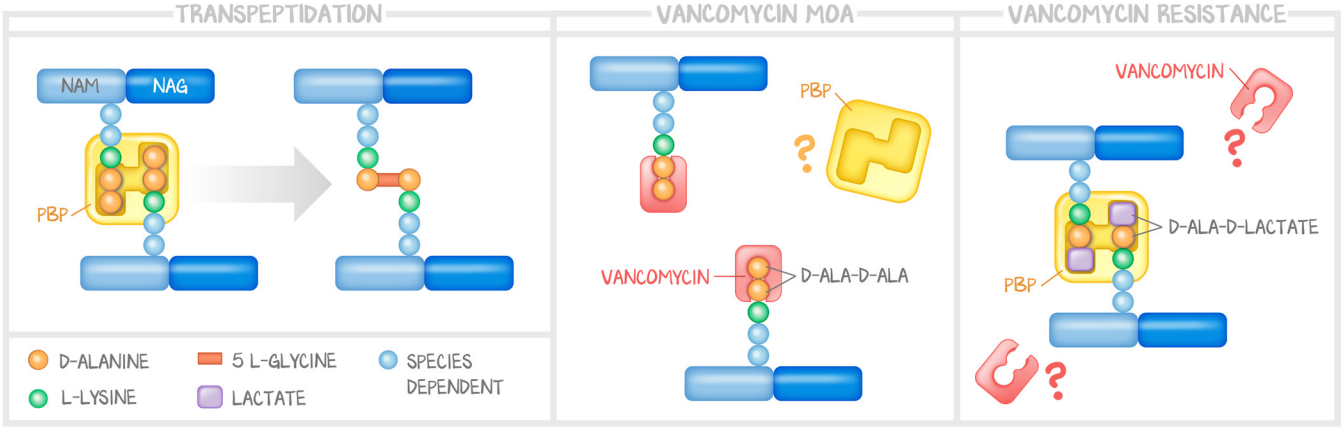

Figure 3.2: Vancomycin Mechanism and Resistance
D-Ala-D-Ala is recognized by PBPs as what should be transpeptidated. Vancomycin binds to the D-Ala-D-Ala better than PBP, hiding the D-Ala-D-Ala from PBP, resulting in failure of transpeptidation. Vancomycin resistance develops from gene mutations that lead to D-Ala-D-Lactate. Because vancomycin has a higher affinity for D-Ala-D-Ala than PBP, it inherently necessitates a lower affinity for D-Ala-D-Lactate. Vancomycin cannot identify D-Ala-D-Lactate, so PBP escapes vancomycin's effects.

But *Staph. aureus* is a clever bug. And we didn't learn from the mistakes of our fathers. Poor antibiotic stewardship (empirically throwing around vancomycin too often) resulted in the breeding of **vancomycin resistance**. But it didn't start in *Staph. aureus*. We were giving vancomycin systemically to treat MRSA infections. But when you expose all bacteria to an antibiotic, any one of them can develop resistance to that antibiotic. Vancomycin resistance first developed in Vancomycin-Resistant Enterococcus (VRE). Vancomycin resistance is conferred by a transposon (see Bacteria #2: *Bacterial Genetics* for details). The **vanA** gene is on **Tn1546.** The gene codes for a simple modification in transpeptidation. VRE figured it out. MRSA figured out how to take an antibiotic resistance gene from another species. Penicillin-binding proteins can recognize D-Ala-D-Ala OR D-Ala-D-Lactate.

Vancomycin cannot. The increased specificity for D-Ala-D-Ala vancomycin has over PBPs inherently means it will have a decreased affinity for some other structure. Vancomycin has a much lower affinity for D-Ala-D-Lactate than PBPs' affinity for D-Ala-D-Lactate.

The alteration of the terminal amino acid of the peptide chain from **D-Ala-D-Ala** to **D-Ala-D-Lactate** rendered vancomycin useless. MRSA became VRSA—resistant to all **β**-lactams and vancomycin. We have a few tricks left up our sleeves, but we end this story here with the lesson of failed antibiotic stewardship. Bacteria develop resistance faster than we can develop new drugs.

Vancomycin is **renally cleared** and must be **dose-adjusted** for renal failure. In practice, troughs are measured to ensure adequate dosing—before every third dose, levels are assessed and dose adjustments made based on the level. High vancomycin levels can cause renal failure, and vancomycin levels will rise in response to renal failure. Vancomycin used to cause **red man syndrome**—give vancomycin, the patient turns red. That reaction was secondary to the formulation, not the medication. Current formulations of vancomycin do not cause red man syndrome. However, if a patient is receiving vancomycin on the exam, and they turn red, pick vancomycin.

Vancomycin **does not cross any blood barrier**. Because it cannot cross the gut-blood barrier, it makes an ideal solution for intraluminal infections such as *C. diff* colitis. **Oral vancomycin** treats C. diff **infections**. Oral vancomycin is not absorbed and so cannot be used to treat anything else. *C. diff* colitis is a Gram-negative anaerobic bacterium. There is no reason why you would reason your way from *Staph. aureus* to *C. diff*. Empiric evidence has proven that oral vancomycin is superior to other treatments of *C. diff*, which makes it prime testing fodder.

Intravenous vancomycin is used to treat **systemic infections** of MRSA. It is the empiric coverage of choice for health care-associated pneumonias, abscess-forming skin infections, osteomyelitis, and endocarditis. If ever an organism is cultured and there is an option other than vancomycin, especially nafcillin, do not choose vancomycin. Given the rise of penicillin-resistant *Strep. pneumoniae*, vancomycin is now also indicated in the **empiric treatment for meningitis** (along with steroids and ceftriaxone). The inflamed meninges allow penetration of vancomycin into the CSF. Under normal conditions, vancomycin does not cross the blood-brain barrier.

Bacitracin

β-lactams (through PBPs) and vancomycin (by binding D-Ala-D-Ala) inhibit cell wall synthesis by targeting the final assembly of the peptidoglycan layers—transpeptidation. Bacitracin is a topical antibiotic that binds to and inhibits the dephosphorylation cycling of the phospholipid carrier (you have not encountered those words before in this course and you will not see them again; included for thoroughness) that transfers the NAG-NAM amino acid from the cytoplasm to the extracellular space. It **DOES inhibit peptidoglycan formation,** but not by interacting with PBPs. No NAG-NAM amino acid, no peptidoglycan cell wall. It is effective against Gram-positive organisms and is used only **topically**. Do not use bacitracin in wound infection prophylaxis. It is available over the counter and is used on cuts and scrapes to prevent infection.

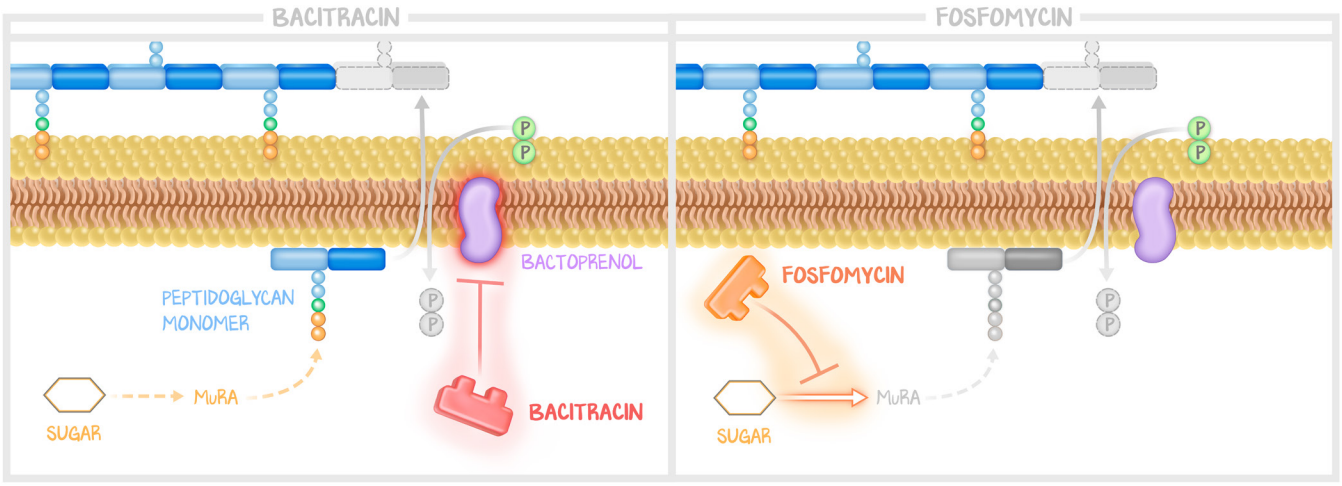

Figure 3.3: Bacitracin and Fosfomycin
(a) The mechanism of bacitracin is to inhibit bactoprenol, preventing the translocation of the NAG-NAM pentapeptide from the cytoplasm to the extracellular space. (b) The mechanism of fosfomycin is to inhibit the initial step in NAG and NAM synthesis, inhibiting the formation of MurA.

Fosfomycin

Fosfomycin also **inhibits peptidoglycan formation** by inhibiting the first enzymatic reaction in the formation of the peptidoglycan backbone. The enzyme name is not worth remembering (enolpyruvate). It makes **MurA**. MurA will go on to become the NAG-NAM sugar backbone of the peptidoglycan. MurA's real name is not worth remembering (UDP-N-acetylglucosamine).

What fosfomycin is best known for, however, is **single-dose treatment of uncomplicated UTIs**. (It sustains high concentration in the urine for days.) It has also been used in 9- and 21-day treatment (3 g at 3-day dosing intervals) of complicated UTIs and prostatitis, respectively. HOWEVER . . . this irregular dosing pattern is the ONLY context in which it will ever appear on an exam. Moreover, it is **never the empiric choice**. Since it is a cell-wall inhibitor, it intrinsically doesn't work well on Gram negatives (these cause UTIs).

Daptomycin

Daptomycin works by **disrupting the plasma membrane**. Multiple daptomycin particles form a **pore** in the cell membrane, resulting in depolarization (the exact mechanism is not known, but the cells do depolarize). Depolarization of the membrane interrupts DNA replication, RNA transcription, and protein translation. In practice, **daptomycin is a vancomycin alternative** for infections that **are not in the lung** (daptomycin is inactivated by the surfactants in alveolar tissue). Specifically, it is the preferred agent for ***Staph. aureus* bloodstream infections** where vancomycin cannot be used. It is renally cleared, but has liver and muscle side effects—rise in the LFTs, elevation in creatine kinase.

When considering MRSA, use vancomycin. When vancomycin cannot be used, use dapto for blood and linezolid for lung (see Antibacterials #4: *Translation Inhibitors*).

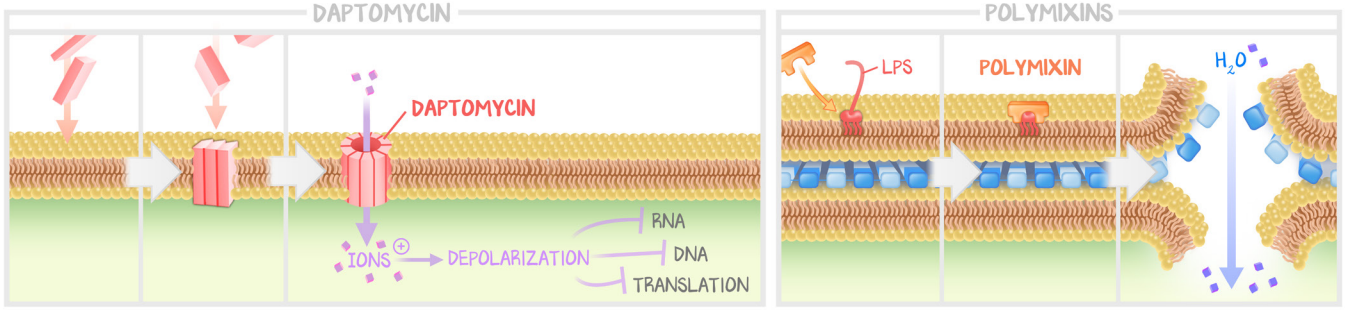

Figure 3.4: Mechanisms of Action, Daptomycin, and Polymyxin B
(a) Daptomycin inserts a channel into the plasma membrane of Gram-positive organisms (it cannot get through the outer membrane of Gram-negative organisms) and causes depolarization, which in turn inhibits all cell functions. (b) Polymyxin B binds to lipopolysaccharide in Gram-negative organisms, which results in loss of plasma membrane integrity. The details are poorly understood.

Polymyxins

Polymyxins are almost never used. Their **nephrotoxicity** often outweighs their clinical benefit. They are **dose-dependent** bactericidal agents that act as a **detergent** disrupting cell membrane integrity. They are useful only in targeting Gram-**negative organisms**, as the polymyxins are activated by **lipopolysaccharide**. Polymyxin B is used as eyedrops against *Pseudomonas* and **polymyxin E** (**colistin**) has been used as an **inhaled antibiotic** to fight *Pseudomonas* in high-risk patients such as those with cystic fibrosis.

Honorable Mention: Telavancin

Somehow this antibiotic has the mechanism of both vancomycin and daptomycin. It binds D-Ala-D-Ala AND punches a hole in the membrane. Telavancin was created for its convenience (no labs to check like vancomycin) and to tackle organisms with reduced susceptibility (but not resistance) to vancomycin. It's a better vancomycin that causes more renal failure than vancomycin. It should be seen only as an experimental antibiotic that should never be used in practice and in the far future, for when *Enterococcus* gets even more resistant than VRE.